The Role of the Connective Tissue Ground Substances (Mucopolysaccharides) in Allergic Injury

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SUMMARY

The basic histologic reactions of the classic allergic diseases and of several systemic diseases in which allergic mechanisms appear to operate are described and illustrated. Particular attention is drawn to the ground substances—mucopolysaccharides—which constitute important elements of connective tissue and vascular structure. The intimate locus of the allergic reaction appears to be in and to involve a swelling of such substances. It is suggested that antibodies (and possibly antigens) may be attached to these mucinous ground substances of the connective tissues.

IT is the purpose of this report to describe and illustrate the character and distribution of lesions in diseases known to be caused by allergic reaction and also in a group of diseases in which allergic mechanisms appear to operate. The discussion will be primarily concerned with the so-called anaphylactic type of hypersensitivity reaction as distinguished from the bacterial or tuberculin type.[19] In an effort to identify a unifying factor in what appear to be dissimilar lesions, particular attention will be directed to the intimate structure of the tissues involved.

THE GROUND SUBSTANCE OF CONNECTIVE TISSUES AND BLOOD VESSELS

In studies of arteriosclerosis[24] and rheumatic diseases, the author's attention was drawn to the substances known as mucopolysaccharides which constitute the ground substance or interfibrillar matrix of connective tissue structures. It is pertinent as a preface to this discussion to describe briefly the character and distribution of the mucopolysaccharides. Such materials are vital structural components of various connective tissues, including blood vessels. They are mucinous in character and serve importantly as mobile cement substances. Only recently have these substances been receiving deserved study.[5, 15] In usual histologic techniques these materials escape attention. In situations where the mucopolysaccharides are concentrated they may be demonstrated by the property of metachromatic

staining with toluidine blue. However, the finely distributed mucopolysaccharides are not well shown by this method. Recently a useful and precise histochemical technique for demonstration of the mucopolysaccharides[23] was developed. It is based upon the method proposed by Hale.[7] In this technique the mucopolysaccharides are stained blue and well differentiated from reticulum and collagen fibers, which stain red, and from basement membranes, which stain an orange yellow. Sites where the connective tissue mucopolysaccharides are found to be present in considerable amounts include the reticular tissue of the skin just beneath the epidermis and surrounding skin appendages, the synovial tissues, tendon sheaths and heart valves. In blood vessels a mucopolysaccharide ensheaths the elastic tissue fibers and lies between the smooth muscle cells of the muscular arteries (Figure 1-A). A similar relationship between mucopolysaccharides and the smooth muscle cells exists in the bronchial wall. Delicate membranes of a mucopolysaccharide surround reticulum fibres and finely dispersed collagen and are in intimate relationship with the walls of capillaries and venules.

The mucins secreted by various epithelial cells are not germane to this discussion, but it should be noted that they are chemically related and assume a similar coloration in the histochemical method employed.

Mechanisms of antibody formation, the sites of antibody and antigen fixation and the precise nature of the injury resulting from their union are still obscure. It is well established that minute amounts of antigenic substance which gain access to the tissue fluids may generate large amounts of antibody which may be found in the blood and, more importantly, become fixed in tissue. Experimental as well as clinical investigations indicate that both sensitization and shock can be elicited by ingestion and inhalation as well as by parenteral administration of antigenic substances.[26]

HISTOPATHOLOGIC CHANGES IN ALLERGIC INJURIES

Urticaria

Evanescent urticarial lesions are characterized primarily by congestion and edema in the delicate reticular tissue beneath the epidermis. This is similar to the early reaction following intracutaneous injection of antigen in a sensitized organism. If the injury due to the antigen-antibody union is more intense and the reaction persists for 24 to 48 hours a cellular response occurs in which the eosinophil is commonly prominent (Figure 1-B). A swelling of the mucopolysaccharide at this site is demonstrable in appropriately stained sections.

Allergic rhinitis

This reaction is characterized by excessive serous and mucous secretion of the nasal mucosa with edema of the submucosal tissue. It is well known that smears of such nasal secretions are particularly rich in eosinophils. Pathologists rarely observe specimens from the early phases of simple allergic rhinitis or hay fever but commonly see the edematous polyps that have resulted from repeated allergic insults. Such a lesion is shown in Figure 1-C. The submucosal edema is striking, and the tissue is infiltrated with eosinophils. The epithelial mucopolysaccharide stains intensely blue and a more delicately stained mucopolysaccharide is present in the edematous submucosa.

Eczema

In eczema the pathologic changes, as observed both grossly and microscopically, are, of course, extremely varied depending upon the intensity of the insult, duration, external irritative factors and infection. Initially the process is one of capillary hyperemia with edema beneath the epithelium and at times vesiculation due to accumulation of fluid between epithelial cells. The mucoid ground substance beneath the epidermis is swollen.

Asthma

The pathologic changes in classical asthma are quite distinctive. In the relatively infrequent case that causes death the lungs are emphysematous, and the mucus which fills the small bronchi is extremely tenacious. The microscopic changes are varied in intensity, but the basic changes commonly observed are mucous plugs in bronchi, overactive mucous secretion of the epithelium, some hypertrophy of the bronchial musculature, and hyaline thickening of the basement membranes. There is a variable mucinous edema and inflammatory cell infiltration of the bronchial wall in which eosinophils are frequently prominent (Figure 1-D).

In consideration of allergic rhinitis, eczema and asthma it is of interest to recall that sensitization of local areas can be established if antigen is concentrated in those areas. One condition favoring such concentration is the circulation of antigen at a time when a local focus of inflammation is present.

Serum Sickness—Sulfonamide and Periarteritis

The syndrome of serum sickness affords a good example of a more generalized hypersensitivity reaction. The major clinical manifestations are well illustrated by the following case record:

A white male 28 years of age had received tetanus antitoxin for a laceration of the hand. In a few hours urticaria developed, then subsided. Two weeks later the patient became acutely ill, with fever, urticaria, painful swollen joints and edema about the eyes. During the ensuing week the clinical manifestations noted continued, and he was admitted to the hospital one week after the onset of symptoms. Upon physical examination, an injected pharynx and diffuse soft swelling of the neck were noted. Several joints were painful on movement and were swollen and reddened. The skin was hot and dry, and on it were urticarial lesions quite like the erythema marginatum of rheumatic fever except that they were more prominent. (It is of interest that Holt and McIntosh,[*] commenting on the occurrence of erythema marginatum, stated that "unless this occurs as a manifestation of serum disease it is nearly always rheumatic in origin.")
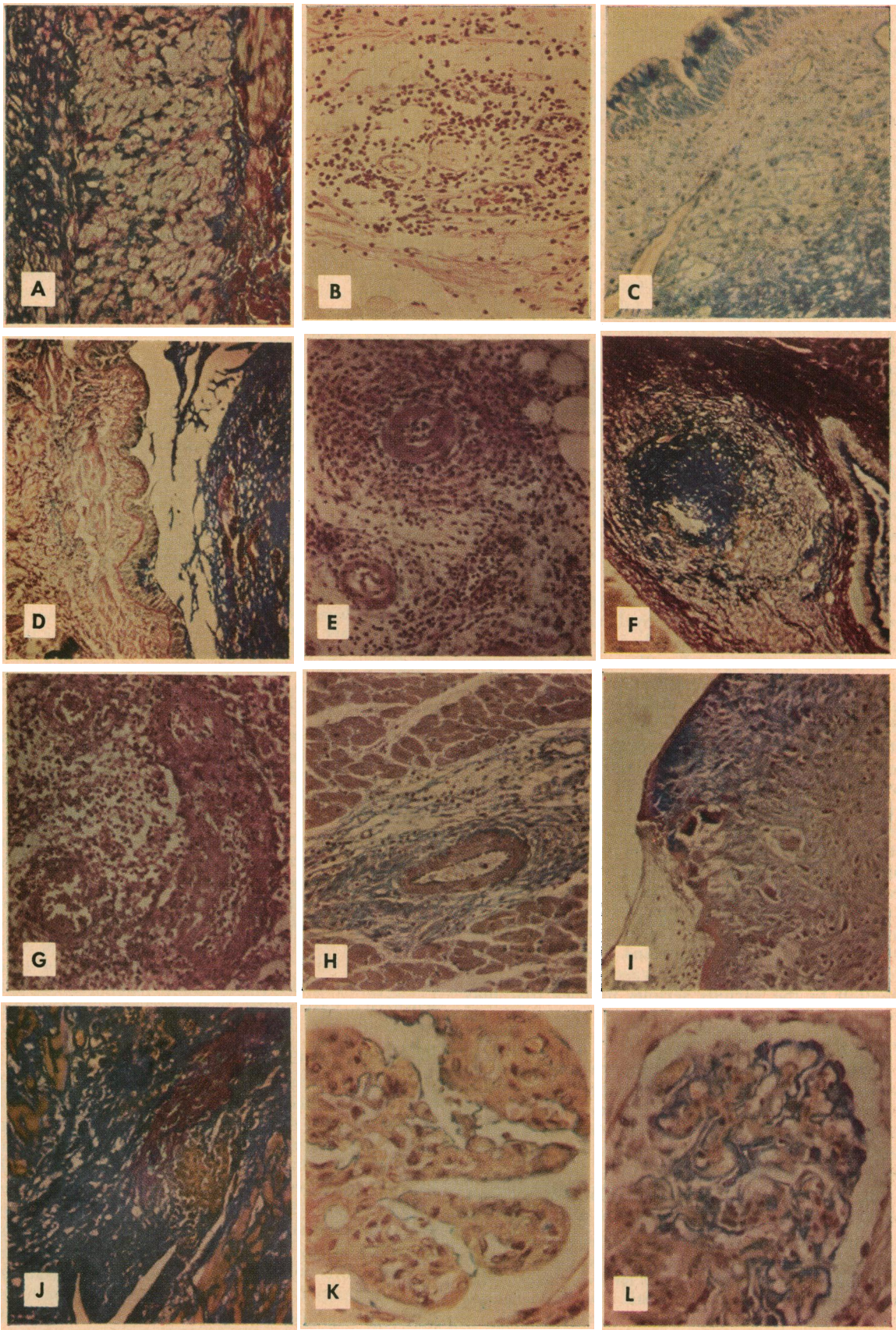
The pulse rate was 110; the blood pressure 120 mm. of mercury systolic and 72 mm. diastolic. Heart sounds were normal. The hemoglobin content and the number of erythrocytes in the blood were within normal limits. Leukocytes numbered 15,900 with 90 per cent polymorphonuclear neutrophils. Eosinophils were not present. The ascorbic acid content of the plasma was 0.32 mg. per 100 cc. No abnormality was noted in an x-ray examination of the chest. Large doses of ascorbic acid (1 gm. four times daily) were administered, beginning on the third day of hospitalization. The tempera-

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Legends for Color Plate

A—An artery with changes of mild arteriosclerosis. The slightly thickened intima with excess mucopolysaccharide staining blue is at the left. The media occupies the central segment. Note the finely distributed mucopolysaccharide between muscle cells. The adventitia is composed of collagen (red) and mucopolysaccharide. Stain C.I.[*] ×116. **B**—Twenty-four hour allergic reaction in skin of guinea pig sensitized to horse serum. Note congestion, edema and infiltration with eosinophils. In appropriately stained sections, much mucopolysaccharide may be observed in this tissue. Stain H. and E.[*] ×116. **C**—Segment of nasal polyp in allergic rhinitis. Note blue mucin in epithelial cells and swelling of the ground substance in the underlying connective tissue. This tissue is infiltrated with eosinophils not well shown in this preparation. Stain C.I. ×116. **D**—Bronchus in asthma. Note excess of epithelial mucin in the lumen, thickening of the basement membrane and mucinous edema of the bronchial wall. Stain C.I. ×58. **E**—Acute arteritis in a case of sulfonamide hypersensitivity. Stain H. and E. ×116. **F**—Acute arteritis. Note excess accumulation of mucopolysaccharide in the vessel wall (blue) and so-called fibrinoid degeneration (yellow). The latter is probably fibrin. Stain C.I. ×58.

G—Arthus reaction in larynx with extreme congestion and necrosis of walls of capillaries and venules. Stain H. and E. ×116. **H**—Early Aschoff reaction in acute rheumatic fever. This is characterized by swelling of the ground substance and beginning cellular proliferation. Stain C.I. ×116. **I**—Verrucous nodule on heart valve in rheumatic fever. The heart valves normally have a high mucopolysaccharide content. In rheumatic endocarditis this substance swells; the intimal surface is interrupted and fibrin is deposited on the surface. The cells proliferate and hypertrophy, assuming the characteristic features of the rheumatic reaction. Stain C.I. ×116. **J**—Characteristic lesion of acute disseminated lupus erythematosus in the heart with prominent swelling of the ground substance (blue). A focus of so-called fibrinoid degeneration may be seen (orange-colored) just beneath the collagen (red). Stain C.I. ×116. **K**—Glomerulus in acute disseminated lupus erythematosus. The "wire loop" lesion is characterized by pronounced thickening of the basement membrane. There is also some glomerulonephritis evidenced by endothelial proliferation and duplication of the basement membrane. The surface epithelium has been largely destroyed at this stage. Stain C.I. ×350. **L**—Glomerulus in early proliferative glomerulonephritis. The surface epithelium with its mucoid cytoplasm stains blue; the basement membrane is stained orange. At the lower left, endothelial proliferation has occluded the capillary loops. Stain C.I. ×230.

*Stains: C.I.—Colloidal iron for demonstrating mucopolysaccharides; H. and E.—Hematoxylin and eosin.

ture dropped, and all manifestation of illness subsided in the next four days. (It is not known just what influence ascorbic acid had on the illness. Favorable influences of large doses of ascorbic in rheumatic fever have been reported.[13])

While skin and articular lesions dominate the clinical picture in serum sickness it is well known that significant visceral lesions may result from vascular injury. Since the early investigations by Vaubel[29] and Klinge[12] there have been numerous studies of the pathologic changes occurring in animals subjected to hypersensitivity injury by injection of foreign sera. Rich and Gregory revived interest in this field of study and reported the occurrence of periarteritis[20] and lesions bearing some resemblance to those of rheumatic fever[21] in rabbits subjected to hypersensitivity injury with horse serum. More and McLean[17] recently reported a rather extensive experimental study of horse serum injury in rabbits and cited most of the pertinent literature. In 1937 Clark and Kaplan[3] described essentially analogous lesions in two men who had received large doses of antipneumococcic (horse) serum. The most common form of vascular hypersensitivity injury observed in man is that which has been encountered since the widespread use of sulfonamides in which the antigen is probably a conjugate of blood protein and the drug. Most pathologists have observed numerous cases of this type.

In serum and sulfonamide hypersensitivity the brunt of the injury is borne by blood vessels, particularly small arteries. Lesser lesions may appear as edema of the media spreading the muscle cells apart. More severe lesions are characterized by necrosis of segments of the muscular wall with a perivascular inflammatory reaction. The injuries are varied in extent and intensity. Characteristic lesions may be seen in Figures 1-E and 1-F. A striking swelling of the mucoid ground substance will be noted in Figure 1-F. Aneurysmal dilatations with hemorrhage may or may not occur. Vascular lesions may be rather widely disseminated and consequently the clinical manifestations may point to involvement of several organs or organ systems. Spastic contraction of arteries may contribute to development of vascular lesions. Smooth muscle contraction is characteristic of certain allergic reactions. Reactions to sulfonamide are not exclusively vascular. Allen[1] drew attention to the various serious injuries that may occur in the kidney.

Arthus Reaction

At times an unusually high degree of hypersensitivity may exist in which the hemorrhagic element is prominent as is seen in the Arthus reaction. Here the injury centers in and around about small arterioles and capillaries and is associated with thromboses and hemorrhages (Figure 1-G).

Recently a case of this type, in which the sensitizing agent appeared to be penicillin, was observed:

A middle-aged white adult gave a history of rheumatic fever in childhood. During the ten years preceding the present illness he had had hay fever and for five or six years had had recurrent episodes of polyarthritis. Five months prior to hospitalization he had nephritis with albuminuria and hyper-

tension. He had taken sulfanilamide some time prior to this. Three months after he had nephritis, a febrile illness with polyarthritis, pleuritis and pericarditis developed. The acute manifestations of this illness subsided in three days on administration of penicillin and salicylates. At that time the urine contained albumin, erythrocytes, leukocytes, and casts. In a phenolsulfonphthalein test, there was 38 per cent excretion in one hour. Administration of salicylates was continued and the patient remained in bed for about ten weeks. Three days before the patient entered the hospital, chilliness, sore throat and fever developed. Coincident with this, erythematous and bullous lesions appeared at the site of previous penicillin injections. Penicillin was again administered. Pain and fullness in the right shoulder developed rapidly, the patient had difficulty in speaking, and there was edema of the eyelids with small hemorrhagic ulcers. A few similar lesions appeared on other parts of the body. Death resulted from what appeared to be cardiac failure three days after the onset of the terminal illness.

In a postmortem examination, edema of the epiglottis and hemorrhagic edema of the false cords of the larynx were noted. The periarticular tissue and synovia of the right shoulder were edematous and hemorrhagic. Residues of the previous illnesses included pleural and pericardial adhesions and relatively small somewhat scarred kidneys. In the lesions of the skin, vocal cords and joint there was extreme vascular engorgement and hemorrhage with necrosis and thrombosis of some of the small blood vessels. A characteristic lesion from the larynx is shown in Figure 1G. Accumulation of mononuclear and polynuclear leukocytes around some of the engorged vessels indicated that there was injury and reaction in the reticular tissue around the vessels as well as of the vessels themselves. In fact, the vascular injury may have been secondary to that in the surrounding reticular tissue. The process appeared to be quite like the explosive hypersensitivity lesion known as the Arthus reaction. In the kidneys, healing lesions which were in keeping with an antecedent sulfonamide hypersensitivity injury, were observed.

This case presents several interesting aspects. The patient had a history of illnesses that either were allergic or had allergic implications. The renal disease which had caused significant impairment of function was of a type that probably had resulted from sulfonamide injury five months before. In the author's experience, severe allergic injuries of the vascular type most frequently occur in patients with an "allergic" background, and often with antecedent renal impairment. Thus the hazard of serious allergic injury with foreign sera, sulfonamides, antibiotics and other allergens would appear to be especially great in the person with history of allergic sensitivity, particularly in the presence of renal impairment.

Rheumatic fever

Since the early studies of Swift[27] a considerable body of evidence has accumulated implicating the operation of an element of hypersensitivity in rheumatic fever. Klinge[12] and his associates, and Vaubel[29] and Junghans[10] reported that in rabbits subjected to repeated doses of horse serum, lesions resembling those of rheumatic carditis developed. This work was extended and reviewed by Rich and Gregory.[21] It is likely that an allergic element contributed to the carditis which Rinehart and Mettier produced in guinea pigs by subjecting them to the combined influence of scurvy and infection with beta hemolytic

streptococci.[25] While it is believed that the scurvy conditioned the connective tissues and altered their reactivity, the deficiency also impaired the localization of the infection and probably permitted greater and more prolonged absorption of antigenic substance. Immunological studies[4, 18] indicated heightened antibody responses in the rheumatic subject. It is well recognized that lesions of periarteritis may occur in rheumatic fever.[6, 21] This is one of the evidences implicating an allergic influence in the disease. The rheumatic inflammatory reaction is characterized by a particular type of proliferative reaction in the heart valves and in the reticular connective tissue surrounding small twigs of the coronary arteries. The size and cytologic features of the reactive cells make the Aschoff body quite a distinctive lesion. In its early and fully developed phase there is an excessive accumulation of a mucopolysaccharide. Talalajew[28] first drew attention to mucinous edema at the site of the developing Aschoff body. Such a lesion is shown in Figure 1-H. The verrucal lesions of the heart valves develop at the lines of closure. The heart valves normally contain a high component of mucopolysaccharide. The verrucal lesions appear to involve a swelling of this material accompanied by loss of surface endothelium and the deposition of fibrin (Figure 1-I).

Acute disseminated lupus erythematosus

Another "rheumatic" disease that warrants consideration in this connection is acute disseminated lupus. Characterization of this disease has largely developed from a series of studies by Baehr and Klemperer[11] and their associates at the Mt. Sinai Hospital. The basic nature of this disease is not clear although there is sufficient evidence for strong suspicion that elements of hypersensitivity operate in its pathogenesis. It is a widespread, commonly fatal disease with a predilection for women in the childbearing period. Lowering of the plasma albumin and elevation of globulin reflect a profound metabolic disturbance. Anemia, leukopenia and thrombopenia are frequently present. Erythematous and hemorrhagic skin lesions are commonly present. Particularly characteristic is the "butterfly" erythema across the bridge of the nose. Arthritis and serositis are frequent manifestations. Renal injury is manifest by proteinuria and by the presence of mixed cellular elements and casts in the urine. Recently an element has been found in the blood which causes clumping of leukocytes and degenerative changes in nuclear structure.[8] The pathologic changes are widespread, involving particularly the skin, heart, kidneys, joints, pleural and pericardial surfaces. Degenerative and proliferative lesions often associated with thromboses are observed in many small blood vessels. In the kidneys the characteristic hyaline thickening of the basement membrane which has been aptly designated as the wire loop lesion (Figure 1-K) may develop. In some cases there may also be endothelial cell proliferation in the glomeruli[1] quite like that which occurs in glomerular nephritis. In about 20 per cent of cases the distinctive lesion known as non-

bacterial thrombotic endocarditis is present. Small sterile thrombotic masses occur along the line of closure of the mitral valve and are commonly found attached to the mural endocardium. While endothelial and vascular lesions are widespread there appears to be a degenerative change in some connective tissue sites which cannot be readily explained on the basis of vascular or endothelial injury. Klemperer expressed the belief that there is a physicochemical alteration in collagenous tissue, probably primarily in the ground substance which constitutes the basic lesion. This he called fibrinoid degeneration. Altschuler and Angevine[2] drew attention to the finding of metachromasia (the staining property of ground substance) at sites of so-called fibrinoid degeneration. Studies by the author bear this out. While agreeing that there is probably a basic defect in the interfibrillar element of collagenous structures, the author is of the opinion that the brilliant eosinophilic staining property which characterizes the so-called fibrinoid degeneration is the result of seepage of plasma protein into the tissue and the consequent deposition of fibrin. A characteristic lesion is shown in Figure 1-J. There is a prominent increase in mucopolysaccharide (blue) with an associated focus of so-called fibrinoid degeneration (orange).

Other diseases with allergic implications

There is substantial evidence that glomerulonephritis may be produced by allergic injury.[14] It would appear significant that the cytoplasm of the glomerular epithelium contains a substance which reacts as a mucopolysaccharide by the histochemical technique employed in this study. The basic reaction in glomerulonephritis involves proliferation of the glomerular endothelium (Figure 1-L), and at times proliferation of the glomerular and capsular epithelium. Buerger's disease is commonly associated with superficial thrombophlebitis as well as involvement of the larger arteries, veins and perivascular tissue. The association of this disease with tobacco smoking seems well established. The lesion in temporal arteritis appears to be fundamentally similar to that of periarteritis. There are several suggestive evidences that rheumatoid arthritis may involve allergic mechanisms. One of the evidences is the occasional finding of associated acute arteritis. Mild obliterative endarteritic changes are more frequent. Space will not permit a detailed discussion of these problems.

DISCUSSION

While there is no question of the dominantly allergic nature of urticaria, hay fever, eczema, asthma and the arteritis of serum sickness or sulfonamide hypersensitivity, the extent of the allergic influences in the other diseases noted is not clearly defined. It is of interest that the classic allergic diseases as well as the systemic diseases with prominent allergic implications occur in sites where mucopolysaccharides are normally present, and the reaction involves swelling of these substances. Thus the reactions in urticaria and eczema develop in the subepidermal

reticular tissue which is richly supplied with muco-polysaccharide. In asthma, there is swelling of the subepithelial mucopolysaccharide and of that which lies between muscle cells. In the arteritis of serum or sulfonamide injury, the reaction occurs in the peri-arterial tissue and between the muscle cells of the vessel wall which are normally bound together by a mucoid ground substance. In glomerulonephritis the mucoid material of the glomerular epithelium is in intimate relationship with underlying proliferat-ing endothelium. The sites of dominant injury in acute disseminated lupus erythematosus and rheu-matic fever are sites at which mucopolysaccharide is normally concentrated, notably the skin, synovia, heart valves and the perivascular stroma of the heart muscle. The lesions characteristically involve a swell-ing of these substances. The idea naturally suggests itself that the mucopolysaccharides of the connective tissues may be the intimate locus of the allergic reac-tion. It is possible that antibody (and in some in-stances, antigen) may be attached to the mucopoly-saccharides. Further study will be essential to evaluate this thesis.

The work of Meyer[16] indicates that the exact chemical structure of connective tissue mucins is varied in different sites. It is possible that chemically different mucopolysaccharides have varied affinity for different antigenic substances. The composition and physical properties of these substances are also dependent upon nutritional influences. Rinehart and Greenberg[24] recently showed that pyridoxine defi-ciency in the rhesus monkey led to deterioration and swelling of the mucoid materials in arteries with development of lesions closely similar to those of human arteriosclerosis. Ascorbic acid deficiency im-pairs the formation of the mucopolysaccharide in association with collagen. The author previously presented substantial evidence that nutritional influ-ences, particularly deficiency of vitamin C, may be contributory to the pathogenesis of rheumatic fever.[22] Thus, metabolic conditioning influences may be important in predisposing to allergic injury and modifying the response of the tissues to the injury.

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